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Evolution in Fungus-Growing Ants

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ABSTRACT

The fungus-growers form a tribe of ants, primarily neotropical and exclusively American, entirely dependent on fungi that they cultivate.

In the simplest nest of the species of small size (1.7-3 mm) there is a single garden. Cyphomyrmex rimosus *uses caterpillar or other insect feces for substrate on which to grow a unique fungus of yeast-like cells; brood is kept separate from the garden and the nest is an irregular cavity taken over by the ants. In other species of this and other genera of small ants a cell, some 20-30 mm in diameter, is excavated that contains a garden of vegetal substrate on which a typical mycelium of filamentous hyphæ develops. Brood is kept in the irregularly cellular garden. The ants are monomorphic, do not forage in file and respond to an external stimulus by catalepsis.*

Species of ants of medium size (3-5.5 mm) may form nests with several cells. Substrate is usually vegetal although insect feces may be included. The ants are virtually monomorphic. Some Trachymyrmex *are transitional to the larger species in occasionally foraging for substrate in file and cutting green leaves and flowers.*

Species of Acromyrmex *and* Atta *have the largest and highly polymorphic workers (2-6 mm and 1-14 mm) and the most populous colonies. There is a marked division of labor, the ants forage in file, cut green leaves or flowers for substrate and are aggressive when the nest is disturbed.*

The true culturing of fungi by ants is confined to the New World tribe, the Attini. All of the species known in this tribe are dependent on fungi cultivated in the form of a fungus-garden. A fungus-garden consists of the fungus together with substrate that is carried in regularly by the ants and especially manipulated before being brought into contact with the fungus. Gardens were not found with some of the attine species in the past but there is now no evidence that any of the species in nature is not dependent on fungus cultured for food by it. The fungi have not been identified outside of ant nests and this relationship may be considered to be an excellent example of symbiosis.

Stages in the evolution of the fungus-growing habit have long been sought among other ants. The well-known harvesting ants, such as *Messor* of the Old World and *Pogonomyrmex* of the New World, gather and store seeds or other plant parts in underground chambers. An ant, *Oxyopomyrmex santschii* of North Africa, closely related to *Messor*, creates a nest similar to that of the attine, *Trachymyrmex*, in having a series of underground chambers at varying levels. Were these stored plant remains to develop a mold or fungus growth at damp times of the year, and were this mold to be eaten by the ants, a stage would be created in the evolution of a true fungus-growing habit. This is not known to be the case. Dacetine ants commonly nest in damp, rotted wood and their tunnels may contain fungi. Their delicate mouthparts have been suggested to be suitable for feeding on mycelia but, again, there is no supporting evidence of a feeding or cultivating habit. In tropical America one commonly finds dacetines nesting in proximity to the nests of attines of various genera and having their brood chambers within several centimeters of the gardens but maintaining complete independence.

Fungi may occur on the walls of the cavities of tropical mymecophytes or ant-plants. Ants are consistently associated with these plants and maintain their brood in the cavities. The mycelium may grow luxuriantly on the detritus of the *Crematogaster* ant colony in the cavities or myrmecodomatia of *Cuviera* in Africa and be cropped by the ants. The consensus of opinion, however, is that actual culturing and feeding have not been proven and that the ants crop the fungus to prevent it from filling the cavities in which they nest. It is noteworthy that in none of the tropical American myrmecophytes with suitable cavities (*Tachigalia*, *Tococa*, *Cordia*, *Triplaris*, etc.) have attine colonies been found. The cavities are large enough for nests of *Cyphomyrmex rimosus* and other small attine species that occur in the same localities.

The European ant, *Lasius fuliginosus*, forms a large nest in the form of a sponge of dark carton on which grows the fungus, *Cladosporium* (=*Cladotrichum*) *myrmecophilum*. This is believed to be eaten by the ants but data on culturing are lacking and *Lasius*, a holarctic genus, is well known to have other food habits.

It appears, therefore, that no light can at present be shone on the origin of the fungus-growing habit from what is known of other ants. Among other insects, it is suggestive that the Old World fungus-growing termites and some scolytid beetles feed on a fungus growing on their feces. Scolytid larvæ depend for food on the fungus that grows on the walls of their galleries. Neither in termites or beetles, however, is exclusive dependence on the fungus for food in all stages known to be the case.

Numerous comparative studies point to the constant manuring of the attine garden by all of the ants in a colony. It is possible that fungi first grew randomly from ant feces deposited in particular places and that the ants fed upon these. The chemical qualities of the feces would be such as to limit the variety of fungi. The constant cropping would further tend to restrict the species and give an advantage to those that would grow a luxuriant vegetative mycelium. The continuation of this habit of defecation in particular areas and cropping and feeding on the fungus developing here could lead to culturing.

The next stage would be to add substrate other than the ant's own liquid feces. This was probably the solid feces of other insects such as wood-boring beetles. An advance from these to caterpillar feces, containing small sections of incompletely digested leaf particles, could lead to collecting fallen leaf and other vegetal particles from the forest floor. The cutting of these into smaller pieces to more readily go down the nest entrance or into cells of the garden, the planting of the mycelium, and the cutting of green leaves would be final stages. The female of the higher attines recapitulates several of the stages when she founds a new colony. She leaves the parental nest with pieces of the hyphae from the garden in her infrabuccal pocket, the repository for detritus from cleaning and feeding that is situated off the pharynx. After digging a cell in the soil she ejects the contents of the pocket and manures it with her feces. The hyphae proliferate and a new garden is created. Eggs are laid on it and she eats many of these, thus enabling her to add more feces. Her stored food in the form of wing muscles presumably is exhausted and this leads to egg-eating. Observations in at least *Cyphomyrmex* and *Trachymyrmex* indicate that she may forage to the outside for fresh substrate.

These stages are represented in the attines discussed below. It is noteworthy that no other ants of any kind are known that so clearly are dependent on fungi for food in the first place and, secondly, culture by adding feces or leafy material of any kind to fungi that they may find growing, either on the walls of their nest or elsewhere.

The development of this mutual dependence between ants and their fungi must have taken a considerable time. It also must have originated during a humid period because of the liability to dessication of these types of fungi. The fact that the optimum temperature in the laboratory for maintaining the colonies and pure cultures of the ant fungi is within one or two degrees of 25° Centigrade argues for a tropical origin, where shaded soil temperatures are similar. Conditions in the present tropics are ideal and apparently humid tropical conditions have persisted for a long time in the Americas and elsewhere. All of the genera and the great majority of species are now found in Tropical America and the genera *Myrmicocrypta*, *Apterostigma* and *Sericomyrmex* occur only on the mainland (Trinidad being considered mainland). *Trachymyrmex*, *Acromyrmex* and *Atta* are the sole genera to stray far into the Temperate Zone. Since fungus-growing ants are unknown from the Congo and other Old World rain forest areas, the evidence would favor the origin of the habit in the Americas during a time of climatic or geographical isolation, cold or dryness being as effective barriers as water.

South America was long isolated during Tertiary times beginning possibly some 70 million years ago, a time when other animals such as marsupial mammals were diversifying here. The South American tropical forests may have been the original home of the attines and their fungi and the symboisis may bave become established during a period of the order of magnitude of 50 million years past. Later in the Tertiary, when the isthmian connection was reestablished, Central America could have acquired its attine fauna that now rivals the South American in its luxuriance. The few attine species now occurring on the northern periphery, to Latitude 40° N., would be comparatively recent migrants.

VARIATION IN MORPHOLOGY (Figs. 4, 5)

Differences in morphology within the Attini are sufficiently great so that some 400 forms have been described in over a dozen genera and subgenera. The worker length varies from about 1.7 mm to 14 mm and spinosity and pilosity vary greatly. The small species are monomorphic, the large species of *Acromyrmex* and *Atta* are highly polymorphic, the latter having a soldier caste.

Most of the species have a sculptured integument and are dull in appearance to the naked eye. Many are densely hairy (*Apterostigma* and *Sericomyrmex*) and all have tibial, tarsal and antennal pilosity. The coarsely tuberculate integument of *Trachymyrmex* and *Acromyrmex* commonly bears curved stiff hairs. Several genera of small species contain ants having smooth contours with sparse pilosity that may consist of scale-like hairs.

The nature of the integument is here emphasized, (1) to illustrate the difficulties in maintaining flourishing cultures of the ant fungus, since spores of alien fungi and bacteria inevitably fall upon the ants, (2) and to contrast with the similarities in behavior.

GENERAL PATTERN OF BEHAVIOR

GROOMING (Figs. 1, 2)

Throughout the range of genera there is fundamental similarity in behavior. One of the activities of peculiar importance is grooming or care of the integument, whether by the ant of its own integument or assisted by other members of the colony. The details of grooming appear to be similar among all species.

In grooming itself an ant uses the pecten at the end of the tibia of each fore leg, drawing the legs of the opposite side between the pecten and the curved, adjacent first tarsal segment. The near surface of the latter bears a row of dense, short bristles which together with the pecten make an effective comb. The funicular segments of the antennæ are kept constantly cleaned in the same manner. The legs and antennae are also drawn between the partly opened mandibles and the other mouthparts, thus cleaning them more thoroughly and moistening them with saliva. Detritus from this cleaning tends to collect in the infrabuccal pocket off the pharynx. The body in general is scraped with the short dense bristles of the ventral surfaces of all tarsal segments and the total effect is to keep the ant externally clean.

As though this were not enough, however, the ants spend much time cleaning one another. In cleaning one another, the one being cleaned remains passive while one or more members of the colony go over it thoroughly with their sub-mandibular mouthparts. The mandibles may be opened or closed and are not used. The hypopharynx of the groomer is appressed to the integument and is applied thoroughly to irregular crevices as well as to flat surfaces. It is in the form of a minutely ridged swab that is moist with salivary secretions.

The sexual forms, and especially the queen, are thoroughly cleaned by the workers and this care may be extended to the wings. The queen remains in one place for long periods. In *Atta* she is so bulky and large as to be incapable of cleaning all parts of her own integument. The smallest workers go down into her narrowest crevices, such as between the head and the thorax, and lick them carefully.

The amount of time spent in grooming depends upon the "dirtiness" of the environment to which the ants are exposed but this is always a major activity. What is accomplished by this?

In the first place, the antennal funiculi appear to be the primary sense organs and must be immaculate to be used efficiently. The terminal segments are continually plied over the substrate and the fungus and actually touch them or come extremely close. They obviously perceive substrate as being suitable for incorporation into the garden and can perceive alien fungi from a variable distance of several millimeters, depending presumably on their pungency.

In the second place, foraging ants come into contact with a host of organisms such as alien fungi, bacteria, protozoa and parasites that would be harmful if taken into the nest and must be removed. Thirdly, the ant integument may at the least be a passive if not nutritive site for organisms that might proliferate in the constantly humid air in and about the garden. Living close together, as these social animals do, requires perhaps more constant care of the integument than in solitary animals and to this has been added their dependence on a fungus susceptible to contamination.

CARE OF BROOD (Fig. 3).

The care of the brood is similar among the attines. The brood is kept in the cells of the gardens, except in *Cyphomyrmex rimosus* where the brood is roughly segregated from the garden and in piles according to size.

It is significant and striking that the brood in all genera known is commonly coated with the mycelium of the fungus. In *Cyphomyrmex rimosus*, where the fungus is cultivated in the form of masses of yeast-like cells, the brood is covered with filamentous hyphæ as in the other attines. This mycelial covering may be so dense as to completely obscure the larvæ and pupæ. The eggs may bear less numerous tufts of the mycelium. The mycelium is planted by the ants on the brood integument as they similarly plant the substrate. For this reason, and apparently because of changing luxuriantness of the fungus in the garden and feeding habits, the brood within a colony may vary in the completeness of its mycelial coat from day to day. The hairy larvæ of *Acromyrmex octospinosus* may lack a fungus coat. All stages of brood among attines are frequently licked by the workers. The saliva as added may be nutritive for the fungus and of course the latter may be food for the adults.

Larvæ are fed by the worker bringing in masses of the fungus and placing them on the mouthparts, where the larva takes over and manipulates the fungus so that it can imbibe the contents of the rasped or punctured cells. It usually rests on its dorsal or lateral surfaces with mouthparts directed outward and frequently embedded in the garden as so much substrate, being held in place by its hairs or by a mesh of mycelium.

The soldiers of *Atta* commonly stay in the nest. They guard the entrances and have been known to stand day after day facing a developing female pupa. When the nest is disturbed they come out and attack the intruder.

CARE OF GARDEN (Figs. 1, 2, 8).

Care of the garden, like grooming, is a constant activity of attines. In the simplest garden, that of *Cyphomyrmex rimosus*, the ants plant the fungus growths or bromatia[1] of yeast-like cells on suitable prepared substrate. The substrate, insect feces in this case, is prepared by licking it thoroughly and continually applying the antennal apices to it. Frequently the ant defecates on it. The bromatia, one fourth to one half millimeter in diameter, are then planted and these are examined from time to time with the antennæ. An ant may pick up one, rotate it between the mouthparts, licking it all the while, then place it back in the original place or at another site. The ants may defecate on these masses as on substrate. The ants may temporarily form a pile of bromatia away from the substrate and these may sprout short hyphæ. Both types of growth are licked. Isolated piles may be of two types, one consisting of the same amber, rounded masses that are usually found on substrate, the other of opaque, grayish white bromatia from which hyphæ are sprouting that are more angular and have not recently been manipulated between the mouthparts.

The gardens of other species and genera are in the form of pellets of substrate covered by filamentous hyphæ. In *Apterostigma* regularly, and to a variable extent in the genera of small Attini, the pellets are insect feces as in *Cyphomyrmex rimosus*. Other attines and especially *Acromyrmex* and *Atta* use fragments of leaves. Before incorporation into the garden the substrate is examined with the antennæ and licked if dry, or bitten around the edges if it is a green leaf section. Frequently the particle receives a fecal droplet.

When thus prepared the substrate is pushed and moved slightly back and forth as it is forced into place, the ant using its mandibles and fore feet. The next stage (Weber, 1956c) is the planting with tufts of mycelium. Tufts are picked up from the nearby growths and placed on the moistened new substrate. An exposed surface of hardly more than one square millimeter may bear ten newly planted tufts within a few minutes. By this habit the growth of fungus is greatly accelerated. The average garden nearly fills the cavity in which it is contained and, when not completely suspended on rootlets permeating the space, it commonly is suspended on pebbles or rock fragments from the floor.

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[1] W. M. Wheeler coined the term *bromatia* in 1907 for the form of the fungus eaten by the ants. The term *myrmecomyces* or "ant-mushroom" (plural *myrmecomycetes*) suggested by my colleague, Professor Lucius Shero, might be introduced as a generic term for the fungi and *staphyla* (plural, *staphylæ*), from the Greek for a cluster of grapes, for the clusters of inflated hyphæ (Fig. 1). These clusters are characteristic of the fungus in the gardens of higher attines and develop similarly in artificial cultures. Staphylæ would be equivalent to bromatia but allude to the form rather than the use. This term was first used in Weber 1957b.

ficance in a normal garden. That it can take place, however, may be illustrated by two observations in artificial nests.

In one case a small amount of the artificially-cultured fungus of *Apterostigma mayri* was placed on the fungus of a garden of *Cyphomyrmex costatus*. The ants immediately recognized it as alien and soon removed and discarded it. Both fungi are Basidiomycetes. In the second case, ants of *Acromyrmex octospinosus* were placed in a tube containing an agar culture of *Atta sexdens* fungus that was contaminated by Ascomycetes of the usual *Penicillium-Aspergillus* types. The ants fed upon the *Atta* fungus as they did consistently on other occasions. One ant finally approached white sporangia of the contaminant and pulled on the long thin stalks. It kept its mouthparts retracted and did not at any time taste this fungus. Frequently it stopped during the weeding and cleaned its fore tarsi and antennæ as these ants do when in contact with unacceptable fungi.

The more significant factors in maintaining a luxuriant growth of the ant fungus are believed to be the secretions added by the ants and the continual care they bestow on the garden. The actions of the female in creating her initial garden have long been known and have been most effectively studied by Autuori (1942). For the colony as a whole, the worker ant feces were shown to be regularly used in the garden (Weber in Wheeler 1937, p. 14, Weber 1941–1956). At one time (Weber 1947, p. 150) the view was held "that the chemical environment created by the malaxated substrate and the ant fecal droplets favor the particular fungus grown by the ants." This view must be modified to include the ant saliva, although this is implied by the word malaxated, and the behavior of the ants. Antibiotic activity of the saliva and feces is indicated. A bacterial flora, present in the succulent substrate, contributes in all probability to the totality of factors working towards a flourishing culture of one fungus. Ant fungi themselves show little or no antibiotic activity against standard test organisms.

NESTS (Figs. 6–11)

The simplest nest is made by *Cyphomyrmex rimosus* and consists of an irregular cavity in the soil or rotted wood, in a curled-up leaf, or in similar sites of constantly high humidity and even temperature.

Other small species characteristically have a crater entrance to their nest in the soil, below which is a single cavity containing the garden. The crater entrance may be erected in the form of a turret or chimney. Species in several genera may have more than one garden. The species of *Apterostigma* usually nest in rotted wood or below this in the soil and rarely form a nest entrance in the form of a crater. More commonly the entrance is a bare hole. Some of the species of this genus have several gardens, each in a separate cell. In general, the species of small size have small colonies (one or two hundred) and small gardens. This is in contrast to many non-attine ants of small size that have large colonies of thousands of individuals (e.g. *Solenopsis*).

The largest species, those of *Acromyrmex* and *Atta*, have the largest nests and have many thousands of individuals in the colony. Commonly the nest contains several gardens that are far larger than in smaller attines. There is more flexibility in the nests of *Acromyrmex* than in *Atta* and the former may nest in debris up in trees, such as humus and decayed vegetation at the bases of bromeliads, or in masses of decaying vegetation at the bases of trees. When they nest in soil they have typical crater entrances.

The large colonies of *Atta* start with a single, small crater entrance. Below this is a single garden containing the female and her first brood. As the colony grows, new chambers and new craters are formed. Scores of chambers, each with its garden, and scores of craters are present in the large, mature colony.

FORAGING, DEFENSE OF NEST AND OTHER BEHAVIOR (Fig. 12)

The species of small size forage singly for substrate and move slowly. At the least disturbance they react by catalepsy, becoming immobile in a standing position. In *Cyphomyrmex rimosus* and other species they may respond by drawing the appendages close to the body and assume a curled-up position. The small ants do not attack a large intruder.

Some ants of medium size, such as *Trachymyrmex septentrionalis*, may forage in file and, upon being disturbed, will attack. These also may cut green leaves and represent in general a stage intermediate between the smallest and largest species.

is a common experience in excavating these nests to have the *Atta* soldiers attack with such effect as to cut the human skin freely to the point of bleeding, to cut holes in woolen socks and even to cut half-moon sections out of leather shoes. Ordinarily the soldiers stay in the nest. Some stand guard facing the queen or immature female stages with great constancy.

Towards intruders of comparable ant size all species may show hostility. The attack of *Cyphomyrmex rimosus* on the much smaller *Wasmannia* ants has been noted (Weber, 1955c). Attine ants, however, are generally characterized by their non-aggressive behavior and all sizes may forage freely by *Pheidole*, *Solenopsis*, *Eciton* and other active ants without hostility. The common *Ectatomma ruidum*, a ponerine ant, has been observed systematically raiding a file of *Acromyrmex* ants carrying pieces of papaya fruit (Weber, 1945). An uncommon behavioral reaction is the "jerking" behavior *Cyphomyrmex costatus* may show. When disturbed, these ants may respond by jigging up and down in a vertical plane, holding on to the garden with the hind feet and lifting the fore feet into the air. It appears to be a response that may tend to alert the colony. Attines do not appear to notice the myrmecophiles, such as roaches and mites that inhabit some colonies. On the other hand, *Acromyrmex octospinosus* ants have been observed to jump with outspread mandibles at white collembolans (*Cyphoderus inæqualis*) infesting their observation nest.

Species of several genera or species within the same genus nest forage and nest freely beside one another without evoking hostility. The large nests of *Atta* commonly have nests of *Trachymyrmex*, *Cyphomyrmex* and other genera in the soil beneath their craters.

Within the genus *Trachymyrmex* differences have been noted repeatedly in observation nests under similar conditions and at the same time. When the nest cover was removed, *urichi* and *zeteki* stayed in the nest, *cornetzi* ran fast and attempted to escape, and *septentrionalis* and its subspecies *seminole* were intermediate in behavior.

EVOLUTION INDICATED BY MORPHOLOGY

There are no known fossil attines and phylogeny of the group itself, based on the modern adults, is at present speculative. Within the tribe, however, the existence of genera of small, monomorphic workers and genera with species of highly polymorphic workers suggests an evolutionary sequence as follows:

Cyphomyrmex rimosus—monomorphic; squamiform, appressed hairs; large, widely-spaced frontal lobes; smooth body contours; small size (about 2 mm).

Cyphomyrmex, other species— monomorphic; pilosity sparse and usually of simple hairs; large, widely-spaced frontal lobes; smooth to spinose body; small size.

Mycetophylax—monomorphic; pilosity sparse; smooth body contours; frontal lobes of medium size and spacing; small size.

Mycocepurus—monomorphic; pilosity sparse; spinose; small size; frontal lobes approximated and small.

Myrmicocrypta—monomorphic; tuberculate thorax bearing squamiform hairs; frontal lobes approximated and small.

Apterostigma—monomorphic; abundant flexuous hairs; smooth body contours; small to medium size.

Sericomyrmex—monomorphic; abundant flexuous hairs; tuberculate body; cordate head.

Trachymyrmex—monomorphic to slightly polymorphic; tuberculate body with stiff, hooked hairs; small to medium size species.

Acromyrmex—polymorphic; abundantly tuberculate body with stiff hairs.

Atta—polymorphic with maxima caste in form of soldier; few and large spines or tubercles in media to maxima castes.

Based on body form of the worker, the most primitive attines may be the *Cyphomyrmex*—*Mycetophylax*—*Mycocepurus*—*Myrmicocrypta* types and the most specialized are likely the *Acromyrmex*—*Atta* group with *Trachymyrmex* clearly transitional. *Mycetophylax* and *Apterostigma* may have diverged independently from this family tree.

EVOLUTION INDICATED BY NEST AND GARDEN (Figs. 6–11)

The nest and garden of *Cyphomyrmex rimosus* as described above, are of the simplest type known. Ants of other species of *Cyphomyrmex* and frequently of other genera of small workers form one symmetrical cell, commonly in the soil, that contains the garden.

The garden itself is fundamentally alike in all known attines except *Cyphomyrmex rimosus*. That of some *Apterostigma* is aberrant in being enclosed in a veil but is otherwise of the general plan. Differences are clearly due to size of ants, small ants having gardens with small cells.

The nature of the substrate, however, suggests an evolution from use of insect feces only, to feces and debris such as bits of leaves and woody material, to these materials plus green leaves or fresh flowers and finally to green leaves and flowers only. The major steps are represented by the following:

Cyphomyrmex rimosus
Small genera and *Apterostigma*
Trachymyrmex
Polymorphic genera

EVOLUTION INDICATED BY BEHAVIOR

The genera of ants that become immobile when disturbed contrast with those that are aggressive when exposed to the same stimulus. *Cyphomyrmex*, *Mycetophylax*, and *Myrmicocrypta* become immobile; *Apterostigma*, *Sericomyrmex* and *Trachymyrmex* usually become immobile but *T. septentrionalis* may show hostility and attempt to bite one when the nest is being excavated. By way of contrast, the ants of *Acromyrmex* and *Atta*, as noted above, regularly attack when one excavates their nest. This suggested evolutionary sequence, again from the genera of small ants to those with polymorphic and large castes is not strictly according to size of the ant. Large ants of *Apterostigma* respond quite uniformly by catalepsy.

Foraging singly and moving slowly, features of the small genera, contrast with foraging in file and moving faster in the polymorphic genera. Again, *Trachymyrmex septentrionalis* is transitional in foraging singly most of the time and in file occasionally. Ants of this genus can run rapidly as in the polymorphic genera. The polymorphic genera frequently forage at night and *Sericomyrmex* and *Trachymyrmex* may do so at times.

The primitiveness of *Cyphomyrmex rimosus* is suggested by the fact that it alone keeps the brood away from the garden. This, however, must be governed by the fact that a garden of wet, exposed insect fecal pellets would be a poor site for brood. In the other ants the same substrate would be insulated from the brood by a mycelial growth.

EVOLUTION OF THE ANT FUNGI (Figs. 1, 8, 10)

The two clearly identified ant fungi are Basidiomycetes, *Lepiota* n. sp. cultivated by *Cyphomyrmex costatus* and *Pholiota* (= *Rhozites*) *gongylophora*, cultivated by *Acromyrmex* and *Atta*. Neither species is known to occur except in association with ants but both are well known genera, the other species in them being unrelated to insects in their occurrence in nature. Such evolution as has occurred in these two ant fungi appears to have been in the loss of the sporophore and consequent dependence on vegetative reproduction through the agency of the ants. The vegetative mycelium has evolved hypal swellings that are advantageous for ant feeding. In *Lepiota* n. sp. the hyphæ are merely enlarged, in the *Pholiota* the hyphæ have developed terminal pyriform to globular inflations and in some cases slight pre-apical swellings. These inflated hyphæ tend to occur in clusters (staphylæ) both in artificial culture and in the ant garden. The greater specializations of the *Pholiota* hyphæ suggest that this fungus is more highly evolved towards symbiosis with ants than is *Lepiota*.

Inflated hyphæ of similar form and in similar clusters occur in gardens of at least some species of *Sericomyrmex*, *Trachymyrmex*, *Acromyrmex* and *Atta*. This suggests parallel evolution of the fungus with these highest ant genera.

The yeast-like fungus of *Cyphomyrmex rimosus* is unique and, since it is unidentifiable, nothing can be suggested as to its origin. The fact that it can also grow in a filamentous hyphal form in artificial culture that resembles somewhat the *Lepiota* may possibly indicate a relationship.

The fungus grown by *Apterostigma* shows abundant clamp connections of the hyphæ and is therefore likely to be a Basidiomycete. The growth of the mycelium as a veil sur-

CONCLUSIONS

From the evidence presented above it would appear that:

A. Ants that culture fungus and depend on it for food are known only from the New World and all belong to the attine tribe (Attini).

B. The stages in the evolution of the fungus-growing habit may bave been:

1. Ants defecated at particular sites in or near the nest.
2. Fungi developed on the feces.
3. The biochemical qualities of the feces restricted the variety of fungi.
4. Certain fungi luxuriated as mycelial growths under these circumstances.
5. The ants cropped these for food, regurgitated this to the larvæ, then in a later stage fed hyphæ to the larvæ.
6. The ants added other insect feces and later vegetal debris, creating a fungus-garden.
7. A vigorous culture of one fungus was maintained, at least partly by antibiotic activity involving the ant feces and saliva that were regularly applied.
8. Chambers were excavated in the soil in which the brood and garden were kept.
9. When females left the parental nest they carried pieces of the hyphæ, either between the mandibles or in the infrabuccal pocket off the pharynx.
10. These fragments of hyphæ were deposited in the cell excavated by the female.
11. The female defecated on these, starting a new cycle of fungus culture.
12. Substrate was added, the luxuriating fungus was fed to the developing brood and a new colony was well launched.
13. Green leaves and fresh flowers were cut and used for substrate; the mycelium was planted.
14. The inexhaustible substrate and efficient culturing of the fungus permitted very large colonies with polymorphic workers to be developed.

C. The fungus-growing habit may have originated in the humid South American tropics during the Tertiary period of the order of magnitude of some 50 million years ago.

D. Within the tribe, evolution in morphology of adult workers and females may have been in the following sequence:

Cyphomyrmex rimosus
Cyphomyrmex, other species, and other genera of small workers
Trachymyrmex
Acromyrmex
Atta
Divergent genera include *Mycetophylax* and *Apterostigma*.

E. Evolution in nest and garden may have followed the same sequence, from the simplest nest of *Cyphomyrmex rimosus* with a garden on insect feces to an *Atta* polydomous nest of large size, based on green leaves.

F. Evolution in behavior proceeded as above, from ants responding to their nest being disturbed by becoming immobile and with appendages drawn close to the body to those responding by aggressiveness and attack, from ants foraging slowly and singly, to ants foraging rapidly and in file. Attines became tolerant of one another and species of the same or of other genera nested freely beside one another.

G. The known ant fungi are Basidiomycetes. That of the most primitive ant, *Cyphomyrmex rimosus*, is yeast-like as cultured by the ants and of unknown affinities. The fungus grow by *Apterostigma* is a Basidiomycete. The two clearly identifiable fungi are *Lepiota* n. sp. (cultured by *Cyphomyrmex costatus*) and *Pholiota* (= *Rhozites*) *gongylophora* (cultured by *Acromyrmex* and *Atta*). These have evolved by repressing the sporophore stage and by developing inflations of the hyphæ that are more advanced in *Pholiota* than in *Lepiota*.

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